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## Reproduction in the Sphacelariales: sex is a rare occurrence

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**ABSTRACT:** This review examines the reproduction and life-history of the Sphacelariales, providing a synthesis of much of the key literature and examining whether an alternation of generations is common within the order. The evidence suggests that sexual reproduction is a rare occurrence for most species, which may be a result of meiosis failing to occur in the unilocular sporangium, onset of sexuality failing to occur in gametophytes or because the necessary environmental conditions are absent. One or all scenarios, or another, may apply depending on the species but, in most instances, there is insufficient information to determine this. Asexual reproduction is common in the order and may be by vegetative means, production of asexual spores or by parthenogenesis or ephobogenesis. Temperature and daylength are known to affect reproduction in the order and the suggested critical daylength for production of propagules in *Sphacelaria rigidula* Kützing has been narrowed to fall between 8 and 9.5 h.

**KEY WORDS:** Sphacelariales, life-history, reproduction, alternation of generations, *Sphacelaria*, *Halopteris*.

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### INTRODUCTION

With the ready availability of DNA sequencing in recent years, clarification of algal phylogeny has progressed rapidly. The most recent revision of the Sphacelariales used chloroplast-encoded psbC and rbcL DNA sequences and showed the order was strongly monophyletic and consisted of four families: Sphacelariaceae, Stypocaulaceae, Cladostephaceae and Sphacelodermaceae (DRAISMA *et al.* 2010). Understanding the reproduction of the order, however, remains to be unravelled in spite of the available literature, which is not inconsiderable but contains many gaps. This review provides a synthesis of much of the available work investigating reproduction of taxa within the order and examines the validity of the inference that a life-history with an alternation of generations occurs for the order.

The phylogeny determined by DRAISMA *et al.* (2010) incorporates the traditionally accepted families based on morphology (DRAISMA *et al.* 2002) but excludes the

Choristocarpaceae, which was considered doubtfully placed (PRUD'HOMME VAN REINE 1982; SEARLES & LEISTER 1980), and introduces the Sphacelodermaceae. This paper accepts the delimitation of the four families recognized by DRAISMA *et al.* (2010) and uses the associated nomenclature.

### Sphacelariaceae

**Asexual reproduction.** The Sphacelariaceae consists of six genera: *Battersia*; *Chaetopteris*; *Herpodiscus*; *Sphacelaria*; *Sphacella*; and *Sphacelorbis*. Vegetative reproduction is common throughout the family, principally by fragmentation although this has not been noted in all species (PRUD'HOMME VAN REINE 1982). Some species, e.g. *Sphacelaria biradiata* Askenasy, *S. rigidula* Kützing, *S. tribuloides* Meneghini, also develop secondary attachment discs, which form commonly on stolons and to a lesser extent on rhizoids (PRUD'HOMME VAN REINE 1982; GIBSON 1989; GIBSON 2003). These discs form the basis of new plants upon separation from the parent plant by

fragmentation of the stolon or rhizoid. They can form erect shoots and become attached when they come in contact with an appropriate substratum, although attachment does not always occur. GIBSON (1989; 2003) also noted the secondary attachment discs on first, second and even third order laterals of cultured *S. rigidula* and *S. biradiata*. These did not attach to the culture dishes and, in *S. biradiata*, did not become fertile. It was thought that the detached state might be a cause of infertility, but attempts to attach specimens using a variety of substrata was unsuccessful. The detached state did not prevent *S. rigidula* from becoming fertile. In some species, e.g. *Sphacelaria plumula* Zanardini, turburculous structures formed as well as, or instead of, the secondary attachment discs (PRUD'HOMME VAN REINE 1982). These, also, could behave as ramets.

In *S. rigidula*, GIBSON (1989) observed nodules in cultures kept at 20°C and 14.5 h irradiance. Under these conditions *S. rigidula* deteriorated rapidly, leaving round balls of tissue which could survive for about one month. When transferred to lower temperatures, the nodules could germinate and form new plants. These nodules were quite different from those noted for the same species by VAN DEN HOEK & FLINTERMAN (1968) or from the 'proliferations' described by PRUD'HOMME VAN REINE (1982) for this and other species. The structures observed by the latter authors developed into secondary attachment discs. The nodules noted by Gibson (1989) developed from secondary segments characteristic of the order and formed a thick wall around them. They were able to undergo a prolonged period of dormancy before germinating into a new plant. Gibson (1989) did not observe these nodules in wild plants.

*Sphacelaria* is the only genus within the Sphacelariaceae, indeed, within the order (Table 1) that produces specialised vegetative propagules. These are small deciduous branchlets that detach readily from the parent and grow into an individual of the same generation. Propagules vary considerably in morphology and have been used to distinguish between species (e.g. GOODBAND 1971; PRUD'HOMME VAN REINE 1982; KEUM *et al.* 1995). PRUD'HOMME VAN REINE (1982) and WOMERSLEY (1987) reported they often occurred in greater numbers when sexual reproductive structures were not present but GIBSON (1989) did not find this to be the case for *S. rigidula*, although did for *S. biradiata*. When the propagule detaches, it leaves behind a basal cell that can form new propagules (PRUD'HOMME VAN REINE 1982; GIBSON 1989; 2003). Germination rates of propagules may vary between and within species. In Australia, Gibson found only few germinated for *S. biradiata*, but 75% or more for *S. rigidula*. In the Netherlands, however, PRUD'HOMME VAN REINE (1982) observed many shed propagules of *S. rigidula* in culture, but only occasionally noted any that germinated.

COLIJN & VAN DEN HOEK (1971) demonstrated that propagule formation could be regulated by both photoperiod and temperature. Their work, combined with that of VAN DEN HOEK & FLINTERMAN (1968), showed that propagules of *S. rigidula* formed at warm temperatures (12, 17 and 20°C) and long days (12 and 16 h of light). GIBSON (1989) grew *S. rigidula* under conditions mimicking those of the seasons experienced at her study sites in Victoria, Australia and found propagules formed under all conditions tested, including under the shorter days and colder temperatures of winter conditions; however, these winter conditions ranged from 11 to 13°C and 9.5 to 10.5 h light, essentially overlapping the warm temperatures and long day lengths used by the former investigators. COLIJN & VAN DEN HOEK (1971) did not find propagules in cultures grown under 8 h light. Thus, the critical daylength for this species probably lies between 8 and 9.5 h.

JOHN & DE GRAFT-JOHNSON (1975) also found a temperature effect in *S. brachygonia* Montagne but not a photoperiodic effect. They found propagules formed on *S. brachygonia* under both long (16:8) and short (8:16) day conditions with temperatures of 20-28°C but not lower temperatures.

There is evidence that asexual reproduction also may occur by more specialised means such as parthenogenesis, epheboogenesis or asexual spores, but this is discussed in the next section on life histories.

**Sexual reproduction and life histories.** An isomorphic or slightly heteromorphic, diplohaplontic alternation of sporophytes bearing unilocular structures with gametophytes bearing plurilocular structures and isogamy or anisogamy has long been considered characteristic for the family, but with the recent affiliation of *Herpodiscus durvillaea* (Lindauer) G.R. South with the Sphacelariales (HEESCH *et al.* 2008), this needs to be broadened to include the presumptive, strongly heteromorphic life-history of *H. durvillaea*. Unilocular sporangia were noted by SOUTH (1974) and PETERS (1990) for this species. They terminated in short erect filaments that occurred between longer vegetative filaments. Upon release, spores settled on nearby filaments, on the sporangia, or remained within the sporangia. Spores then developed into minute gametophytes that subsequently transformed, entirely, into gametangia (PETERS 1990). Peters noted release of pale zooids from the gametangia and suggested a heteromorphic life cycle with an alternation of an endophytic, parasitic sporophyte with a minute gametophyte and isogamy. While this life-history is highly probable, the entire sequence has not been definitively demonstrated. The entire sequence of an alternation of generations in the family has been demonstrated in only one species, *S. rigidula*, although evidence suggests this could be widespread in the family.

Table 1. Chromosome numbers in the Sphacelariales

Species	Chromosome number		Reference
	haploid	diploid	
<i>Battersia mirabilis</i>	8		
<i>Halopteris filicina</i>	16	32	Knight 1929
	16	32	Mathias 1935
	8	16	Schwarzenbach 1957
<i>Halopteris psuedospicata</i>	15-16 (wild gametophyte – one count)	30 (wild plant – first generation sporophyte – see text - 1 count)	Gibson 1989
	15-16 (cultured plant grown from parthenogenesis – 2 counts)	30-32 (cultured plant resulting from fertilisation – 2 counts)	Gibson 1989
		30-32 (cultured plant grown from a unizoid – 3 counts)	Gibson 1989
		30-32 (wild plant – second generation sporophyte – see text-2 counts)	Gibson 1989
<i>Halopteris paniculata</i>		30 (wild sporophyte –1 count)	Gibson 1989
		30-32 (cultured plant grown from unizoid – one count)	Gibson 1989
<i>Halopteris scoparium</i>		20-40	Swingle 1897
		32	Escoyez 1979
	16	32	Knight 1929
	16	30	Higgins 1931
<i>Sphacelaria cirrosa</i>		22-24	Prud'homme van Reine 1982
		28-34	Prud'homme van Reine 1982
	12-16		Clint 1927
<i>Sphacelaria plumigera</i>	12		Prud'homme van Reine 1982
<i>Sphacelaria rigidula</i>	25-30	50-60	Van den Hoek & Flinterman 1968
		35-46	Prud'homme van Reine 1982

The life-history of *S. rigidula* in the Netherlands (VAN DEN HOEK & FLINTERMAN 1968) and in Australia (GIBSON 1989) has been determined as weakly heteromorphic, diplohaplontic and anisogamous. In both studies, unilocular structures were produced in cultures kept at colder temperatures: 4°C in Van den Hoek and Flinterman's study and 11, 12 and 13°C in Gibson's study. This reflected respective winter conditions, which is when wild plants are found with unilocular structures (PRUD'HOMME VAN REINE 1982; GIBSON 1989). Only few unilocular structures were produced in the Australian specimens at 11 and 12°C. COLIJN & VAN DEN HOEK (1971) also found unilocular structures in cultures at 12°C while PRUD'HOMME VAN REINE (1982) found them at 4°C. There also appears to be a photoperiodic requirement as unilocular structures

occurred only at the shorter daylengths of 12.5 h or less across the four studies.

These unilocular structures proved to be sporangia as zooids obtained from them developed into gametophytes bearing distinct micro- and macro- plurilocular gametangia; however, the European plants were dioecious (PRUD'HOMME VAN REINE 1982) while Australian *S. rigidula* was monoecious although plants with exclusively or predominantly macro-gametangia were more common (GIBSON 1989). A temperature and daylength influence was demonstrated for the European plants within experiments although some variation occurred between experiments. COLIJN & VAN DEN HOEK (1971) reported female gametangia at 4 and 12°C and 16:8 (light:dark) regimes but not at higher temperatures or shorter daylengths.

VAN DEN HOEK & FLINTERMAN (1968) also reported gametangia under these, but not higher, temperatures at 12:12 regimes although did not specify whether these were micro- or macro-gametangia or both. TEN HOOPEN *et al.* (1983) investigated only male plants at 12°C and found gametangia at daylengths of 14, 16 and 24 (few) h but not less. A temperature/daylength influence was not demonstrated in Australian plants but cannot be ruled out. GIBSON (1989) found gametangia at temperatures from 11 to 20°C and at daylengths from 9.5 to 14.5 h but she did not investigate lower temperatures or shorter daylengths.

Fertilisation of macro-gametes by micro-gametes resulted in diploid sporophytes. Van den Hoek and Flinterman coupled their work with chromosome counts and successfully showed the associated changes in ploidy levels throughout the life-history. Parthenogenesis was noted by both VAN DEN HOEK & FLINTERMAN (1968) and GIBSON (1989). Female gametes could develop into the parent generation or into a haploid sporophyte generation that bore unilocular structures, which did not appear to be fertile and is considered a 'dead end' pathway; however, haploid sporophytes could be maintained within a population by propagule production.

PRUD'HOMME VAN REINE (1982) cultured many species within the Sphacelariaceae but did not succeed in following the complete sequence of a life-history of any species; however, he provided strong evidence to suggest alternation of generations for some. For example, in *Sphacelorbis nanus* (Nageli *ex* Kützing) Draisma, Prud'homme & H. Kawai, he observed that zooids from plants with plurilocular structures, presumed gametophytes, could develop into plants with unilocular structures, presumed sporophytes. He had not observed fertilisation, but this could not be discounted. This suggests an alternation of generations. From the zooids of plurilocular structures, he also noted development of plants that produced plurilocular or mini-unilocular structures, reminiscent of the parthenogenesis noted above for *S. rigidula*. In cultures with only mini-unilocular structures, he subsequently found young plants bearing either plurilocular or mini-unilocular structures, suggesting an asexual pathway; however, he designated this as uncertain in his Fig. 211, a diagram of the hypothetical life-history of the species. Presumably, he had missed seeing the unizoids. Coupling observations from his field collections, those of others, and his culture experiments, PRUD'HOMME VAN REINE (1982) suggested similar life-histories for *Chaetopteris plumosa* (Lyngbye) Kützing, *S. tribuloides* and *S. plumula* as did GIBSON (2003) for *S. biradiata*. She followed the reproduction of a wild population of *S. biradiata* over a 14 month period and endeavoured to parallel this with culture studies. Propagules, unilocular structures and plurilocular

structures were found in the field but cultured plants never became fertile. Macro- and micro-gametangia were found, usually on separate plants, and fertilisation was observed.

PRUD'HOMME VAN REINE (1982) found data was too scarce to suggest life-histories for *S. reticulata* Lyngbye, *Spacella subtilissima* Reinke, *Battersia plumigera* (Holmes *ex* Hauck) Draisma, Prud'homme van Reine & H. Kawai, *Sphaceloderma caespitula* (Lyngbye) Draisma, Prud'homme van Reine & H. Kawai, *B. arctica* (Harvey) Draisma, Prud'homme van Reine & H. Kawai, *B. racemosa* (Greville) Draisma, Prud'homme van Reine & H. Kawai, *B. mirabilis* Reinke *ex* Batters, *S. cirrosa* (Roth) C. Agardhand, *H. sympodiocarpus* (Sauvageau) Draisma, Prud'homme van Reine & H. Kawai, which he also examined.

Certainly, apparent sporophytes and gametophytes have been collected for many species (e.g. SAUVAGEAU 1900-1914; WOMERSLEY 1967; 1987; PRUD'HOMME VAN REINE 1982; GIBSON 1989) and suggest an alternation of generations is possible, but for a number of species, to the best of my knowledge, plurilocular zoidangia are unknown e.g. *S. brachygonia*, *S. plumula*, *S. novae-hollandiae* Sonder, *H. carpoglossi* (Womersley) Draisma, Prud'homme van Reine & H. Kawai, *H. pulvinata* (J.D. Hooker & Harvey) Draisma, Prud'homme van Reine & H. Kawai, *S. lacustris* Schloesser & Blum, and *Spacella subtilissima*. How do these species reproduce? It does not seem it would be by an alternation of generations. *Sphacelaria brachygonia*, *S. plumula* and *S. novae-hollandiae* produce vegetative propagules, so populations easily can be maintained from these; but the other species do not. These produce only unilocular structures, so, reproduction is not sexual. Presumably, new plants can develop from fragments as this appears common throughout the order, and has been noted by PRUD'HOMME VAN REINE (1982) for most of the species he examined. The development of basal discs along stolons and rhizoids also is likely to facilitate vegetative reproduction, especially as this has been noted for species in the Stypocaulaceae and Cladostephaceae as well (GIBSON 1989).

In species which produce only unilocular zoidangia, consideration should be given to the idea that, perhaps, meiosis does not occur and asexual zooids are formed. We have seen earlier that haploid sporophytes are produced in *S. rigidula* and *Spacelorbis nanus*. Zooids of the latter appeared fertile while those of the former did not; however, this may have been an artefact of culture conditions. We do not know what happens in the field. PRUD'HOMME VAN REINE (1982) cultured many species of the Sphacelariaceae in his investigation on the family's taxonomy and he reports some unusual observations. Most species included specimens from diverse geographic locations around Europe. Not uncommonly, he would describe production of zoidangia only after years of

culture under the same conditions, e.g. 5.5 years in one culture and 3 years in another for *B. racemosa*. Specimens of one species would become fertile when collected from one area but not another. Release of zooids from a zoidangium sometimes occurred as one cohesive unit which formed a nodule and produced filaments, rather than as zooids that would separate from each other. Sometimes zooids did not release and would remain within the zoidangium and produce filaments, or not. Then there is the production of tuberculoid structures which could be induced through infection by a chytrid; however, these structures can produce filaments and form the core of a potential ramet.

For many species, PRUD'HOMME VAN REINE (1982) states that zoidangia are rarely reported from collections of wild specimens in Europe, either from his own or those of others. For some species that produce both unilocular and plurilocular zoidangia, he states that one type of zoidangium, usually unilocular, is more common than another. For such species, then, an alternation of generations would be uncommon. Even for *S. rigidula*, which occurs almost worldwide, he states that unilocular zoidangia have not been noted often on European coasts. In Australia, however, unilocular zoidangia are common (GIBSON 1989), much more so than plurilocular zoidangia but the latter are not rare. It is possible that the occurrence of an alternation of generations may occur commonly for a species in one area but not another.

### Sphacelodermaceae

The Sphacelodermaceae is a recently created (DRAISMA *et al.* 2010), monotypic family containing *Sphaceloderma helgolandica* (Kuckuck) Waern, previously known as *Sphacelaria caespitula* Lyngbye. Vegetative reproduction has not been described for this species.

The life-history of this species remains unknown although unilocular and plurilocular zoidangia often have been observed (LUND 1950; IRVINE 1956; PRINGSHEIM 1874; PRUD'HOMME VAN REINE, 1982, SAUVAGEAU 1900-1914). Prud'homme van Reine found zoidangia after nine years of culturing and grew healthy plantlets from uni- and plurilocular zoidangia but these never became fertile. PRINGSHEIM (1874) found plurilocular and presumptive unilocular structures on the one plant. The latter were either very young or empty, thus, could not be distinguished with certainty from empty plurilocular structures (PRUD'HOMME VAN REINE 1982), that had non-persistent walls (SAUVAGEAU 1900-1914). Lund, however, described persistent walls in plurilocular zoidangia and Prud'homme van Reine found that both persistent and non-persistent walls occasionally occurred in the same plurilocular zoidangium. KUCKUCK (1894 in PRUD'HOMME VAN REINE 1982) found unilocular zoidangia and to plurizoidangia

occurred on separate plants. These were stalked. Kuckuck also found non-stalked subspherical unilocular structures as did Sauvageau but Prud'homme van Reine considers these to be caused by a chytridiaceous fungus.

### Cladostephaceae

**Asexual reproduction.** *Cladostephus spongiosus* (Hudson) C. Agardh is the only member of the Cladostephaceae. In culture, GIBSON (1989) noted that fragments excised from mature plants could develop into plantlets but this has not been observed in the field, although theoretically it would be possible. Both GIBSON (1989) and SAUVAGEAU (1900-1914) observed that stolons, formed from peripheral cells of the holdfast and developed attachment discs along its length thus, potentially, could facilitate vegetative reproduction. Vegetative reproduction has not been described in field plants.

A discussion on possible asexual reproduction by zooids is presented in the next section. GIBSON (1989; 1994) reported that zooids from unilocular zoidangia occasionally did not separate upon release. Flagella were oriented toward the outside of the spore mass, which remained motile for 10 minutes before the mass rounded up and behaved as a single disc or holdfast (GIBSON 1989). This has not been reported elsewhere for *C. spongiosus* but, in *S. rigidula*, VAN DEN HOEK & FLINTERMAN (1968) observed the release of a single monospore from unilocular sporangia on several occasions. These did not develop in culture and PRUD'HOMME VAN REINE (1982) suggested they were due to the use of sodium alginate as a mounting fluid, but GIBSON (1989) used fresh seawater.

**Sexual reproduction and life-histories.** SAUVAGEAU (1900-1914) and SCHREIBER (1931) examined the reproduction of *Cladostephus* but their observations appeared contradictory (GIBSON 1994). Sauvageau found that zooids from plurilocular structures could germinate without fertilisation occurring and deemed them to be 'neutral' or asexual spores. Schreiber, however, determined they were isogametes and described fusion of a positive and negative mating strain with the presumptive female gametes rounding up and settling before being fertilised by the presumptive male gametes. Fertilisation was not observed when gametes from only one plant were used and it was suggested that plants were dioecious. Two mating strains also were described by Meinderts (in MÜLLER *et al.* 1986) but her paper described the sexual pheromone of *C. spongiosus* and did not provide detail on the actual sexual process. GIBSON (1989; 1994) conducted fertilisation experiments of fresh material collected every month over a thirteen month period and clearly demonstrated that plants were monoecious but self-incompatible, explaining why Schreiber observed fertilisation only when gametes

of two plants were mixed together. She noted that sexual behaviour and fertilisation occurred for only 2-3 weeks in winter. The rest of the year, zooids from plurilocular structures could germinate without fertilisation occurring. These zooids were positively phototactic while those that showed sexual behaviour were negatively phototactic. A similar onset of sexuality for a brief period in winter also was described for *Colpomenia peregrina* and complanate *Scytosiphon* by CLAYTON (1979; 1980), so Gibson suggested that *C. spongiosus*, similarly, might produce asexual spores for most of the year and functional gametes for only a brief period in winter.

Gibson's work clarifies what appeared to be the contradictory observations of Sauvageau and Schreiber and supports the generally accepted concept (SCHREIBER 1931; FRITSCH 1945; PRUD'HOMME VAN REINE 1982) that *C. spongiosus* is an isomorphic diplohaplont. She suggests, however, that sexual reproduction is rare and that asexual reproduction via plurilocular sporangia borne on the gametophyte is more common. Indeed, WOMERSLEY (1967) stated that plants with unilocular organs did not occur in Australia, indicating that sexual reproduction, hence alternation of generations, did not occur. GIBSON (1989; 1994), however, found Australian plants with unilocular sporangia at one of her sites, Point Lonsdale, Victoria, where she collected on a monthly basis over a 13 month period, but not at her other eight sites although seasonal collections were made at three of them: Flinders, Sorrento and a second population at Point Lonsdale. One could argue that this reflects sampling intensity and that if such sampling intensity is required to locate plants with unilocular structures, it indicates that presumptive sporophytes are rare, hence sex and alternation of generations is rare. In November, however, 60% of plants in her main study population had unilocular structures. This does not constitute rare. In October, December and January, something like 20% of the population had unilocular structures, i.e. one in five plants. This might be uncommon but is not rare. Because of this, it would be unlikely that she would have missed collecting specimens with unilocular structures in her seasonal populations had they been present. Thus it might be that they occur in one area and not another. Indeed, LINDAUER (1947) and LINDAUER *et al.* (1961) described plants with unilocular and plurilocular structures as common in New Zealand but in separate populations (as *C. australis* and *C. verticillatus* respectively).

GIBSON (1989; 1994) did not carry out chromosome counts for either presumptive sporophytes or gametophytes but, as she convincingly demonstrated fertilisation, it is likely that both haploid and diploid plants occur and that an alternation of generations and the production of sporophytes is a possibility. Her Fig. 12 (GIBSON 1994)

indicated that plants with plurilocular structures were most common from May to August (late autumn and all winter). Sexual activity occurred in July (mid-winter). Smaller and younger plants were noted from September. Gibson's suggestion that these plants arose from sexual (a typographical error states this as asexual) activity or parthenogenesis, or that they arose from asexual zooids, is not incompatible with the data.

From all available evidence it would seem that her suggested life-history (GIBSON 1994) is highly likely but, with the observations of LINDAUER (1947) and LINDAUER *et al.* (1961), that populations with only unilocular zoidangia can occur, a further asexual cycle should be added (Fig. 1); i.e. that zooids from unilocular zoidangia can produce more plants with unilocular zoidangia. Further support for this is that it also is known to occur in the Sphacelariaceae, as mentioned earlier.

### Stypocaulaceae

**Asexual reproduction.** The recent revision of the Sphacelariales by DRAISMA *et al.* (2010) identified four genera in the Stypocaulaceae: *Halopteris*, which is the largest with about 16-20 species depending on the authority followed, *Protohalopteris* (monotypic), *Phoiocaulon* (four species) and *Ptilopogon* (monotypic). None of them is known to produce specifically dedicated vegetative reproductive structures as occurs in *Sphacelaria*, but fragments generally are described to grow easily in culture (e.g. MOORE 1951; PRUD'HOMME VAN REINE 1982; GIBSON 1989). Whether they do so in the field is unknown. PRUD'HOMME VAN REINE (1982) describes nodules sometimes forming as proliferations along filaments of *Protohalopteris radicans* (Dillwyn) Draisma, Prud'homme van Reine & H. Kawai, or from zoidangia when release did not occur. These can produce filaments so, potentially, can break off and form a new plant. GIBSON (1989) noted nodulation in older, deteriorating filaments of *Halopteris psuedospicata* Sauvageau and *Halopteris paniculata* (Suhr) Prud'homme van Reine, which were extremely fragile. The nodules resembled those she observed for *S. rigidula* but were quite different from those noted by Prud'homme van Reine. Gibson also noted development of filaments from these nodules. PRUD'HOMME VAN REINE (1982) occasionally noted formation of secondary attachment discs while MOORE (1951) and GIBSON (1989) noted formation of plantlets along rhizoids.

**Sexual reproduction and life-histories.** Species in the Stypocaulaceae are described as having an isomorphic, diplohapontic life-history with anisogamy or oogamy (PRUD'HOMME VAN REINE, 1982; WOMERSLEY 1987). Meiosis has been demonstrated in unilocular sporangia of *Halopteris scoparia* (Linnaeus) Sauvageau (HIGGINS 1931)

and *Halopteris filicina* (Grateloup) Kützing (MATHIAS 1935). It has been demonstrated more recently in the latter species by KATSAROS & GALATIS (1986) who investigated the ultrastructure of its zoosporogenesis and provided a detailed description. Meiosis also has been demonstrated in the unilocular sporangium of *S. cirrosa* (CLINT 1927, as *S. bipinnata*) and *S. rigidula* (VAN DEN HOEK & FLINTERMAN 1968) in the Sphacelariaceae and the unilocular sporangium initial is widely accepted as the site for meiosis in the order (PRUD'HOMME VAN REINE 1982).

SAUVAGEAU (1900-1914) reported two types of plurilocular gametangia in *Halopteris filicina*, *Phloiocaulon* spp. and *Ptilopogon botryocladus* (J.D. Hooker & Harvey) Reinke. They differed in colour and size of their loculi, those of the female being twice as large as those of the male. In *Halopteris scoparia*, he also described reproductive organs of two types, antheridia and oogonia. HIGGINS (1931) and FRITSCH (1945) were not convinced that oogamy occurred. MOORE (1951), however, recorded oogamy in five species of *Halopteris* and described fertilisation and germling development of *Halopteris congesta* (Reinke) Sauvageau. WOMERSLEY (1987) also reported oogonia and antheridia for *Halopteris* species, as did GIBSON (1989) for *Halopteris paniculata* and *Halopteris pseudospicata*. GIBSON (1989) described fertilisation and germling development as well. Each author described species as monoecious with gametangia developed in axillary sori of mixed plurilocular antheridia and unilocular oogonia. MOORE (1951) and GIBSON (1989) both observed antheridia to deshisce apically and release sperm *en masse*. Upon contact with seawater, individuals soon swam actively away. During fertilization, a cloud of active sperm surrounded each egg. In neither study did germlings develop into adult, fertile plants. Gibson, however, coupled her work with chromosome investigations and counted 15-16 chromosomes in wild gametophytes, 30 in wild sporophytes and 30-32 in plants developed from zygotes (Table 1) clearly demonstrating an alternation of generations. These numbers correspond well with those for most other species in the family (Table 1), and order, for which counts have been carried out.

Despite the good evidence for sexual reproduction, however, the rarity of gametophytes is widely recognized (e.g. SAUVAGEAU 1900-1914; LUND 1950; MOORE 1951; WOMERSLEY 1967 and GIBSON 1989) suggesting that a regular alternation of generations may not always occur. Indeed, GIBSON (1989) found only six and two gametophytes respectively for *Halopteris pseudospicata* and *Halopteris paniculata* out of more than 1000 plants examined for each species. MOORE (1951) explained the low number of gametophytes to be due to two things: that vegetative reproduction (presumably of the sporophyte) occurred most of the time and that the gametophyte was

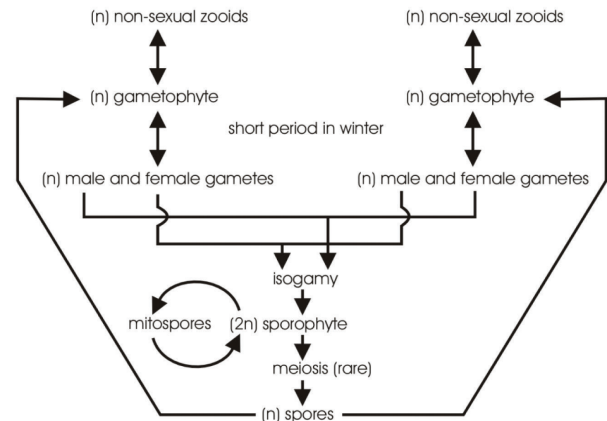


Fig. 1. Life-history of *Cladostephus spongiosus*. As well as what has been drawn, the gametophyte and sporophyte probably can undergo vegetative reproduction as well.

less hardy than the sporophyte. LUND (1950) suggested an alternation of generations once may have occurred but now is completely or almost vanished, and that meiosis in the unilocular sporangium did not normally occur but, instead, asexual spores were produced. LUND (1950) reported that plants of *Halopteris scoparia* bearing unilocular sporangia produced spores that developed into more plants that produced unilocular sporangia as did GIBSON (1989) for each of three wild populations of *Halopteris pseudospicata* and *Halopteris paniculata*, which she followed for a 14-month period. She coupled this with chromosome counts. Numbers for *H. pseudospicata* showed the two successive populations to be diploid (Table 1). Plants grown in culture from spores of unilocular sporangia of the first (parent) population also proved to be diploid. GIBSON (1989) thus has demonstrated without doubt that an alternation of generations does not necessarily occur. Only two chromosome counts were provided for *Halopteris paniculata*, those from the first (parent) population and from plants grown in culture from their spores. This, again, shows a clear case where alternation of generations did not occur and a diploid generation was followed by another diploid generation.

KAWAI & PRUD'HOMME VAN REINE (1998) reported *Halopteris dura* (Ruprecht) Perestenko to have an isomorphic life-history with anisogamy. Unilocular structures did not release in culture but developed erect thalli *in situ*. These thalli produced two types of plurilocular gametangia on separate thalli. Fertilisation was not observed.

*Halopteris filicina* also is anisogamous and an alternation of generations was described by KEUM *et al.* (1995) who demonstrated a temperature effect. Plants



produced unilocular sporangia at 15 and 20°C but not at 10°C. Spores from these developed into monoecious, anisogamous gametophytes, the zygotes of which grew into more unilocular sporangia bearing sporophytes. Field observations of *Halopteris psuedospicata* and *Halopteris paniculata* by MEINDERTS (1984) showed unilocular sporangia were less common in the hotter months and gametophytes were found only in winter suggesting a temperature effect is possible. PRUD'HOMME VAN REINE (1982) noted possible temperature and daylength effects for *Protohalopteris radicans*. At 4°C, low light and short days, plurilocular structures were considered 'not uncommon' in a number of cultures from different countries. Zygotes did not form when gametes from one plant were used but fertilisation experiments between plants could not be performed as gamete release never occurred from two plants at the same time. This could be an indication that an alternation of generations, though possible, may be infrequent for this species.

Parthenogenesis of unfused female gametes in species of the Stypocaulaceae has been observed by PRUD'HOMME VAN REINE (1982) and KEUM *et al.* (1995)

There remains much still to examine concerning the sexual reproduction and life histories within the Stypocaulaceae but here, as throughout the rest of the order, it would seem that sexual reproduction is rare.

**Concluding comments.** Genetic relationships between taxa have clarified the taxonomy of the order, but it would be interesting to determine the genetic variability within species. Low genetic diversity does not bode well for any organism, but especially at a time when our environment faces many human-induced pressures. It is apparent that while an alternation of generations can occur in the Sphacelariales, it generally is rare. The reasons for this are unknown. In *Halopteris*, it may be that meiosis has become rare. In *Cladostephus*, it seems there is a trigger for the onset of sexuality in gametophytes but, in regions where only sporophytes occur and are succeeded by other sporophyte generations, meiosis may not occur. Overlying this are possible temperature, daylength and light quality requirements, which are indicated for the order. Sometimes data appear contradictory between authors, as was the case for *Cladostephus spongiosus* until the full story emerged. The pieces of information that appeared contradictory were shown to be different pieces of the puzzle. Many studies on the life-histories of species within the Sphacelariales are incomplete, but the evidence suggests sex is a rare occurrence in the order. Much work is needed to determine why and requires parallel field, culture and genetic investigations.

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REZIME

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**Ključne reči:** Sphacelariales, životni ciklus, reprodukcija, smena generacija, *Sphacelaria*, *Halopteris*.